THE YALE JOURNAL OF BIOLOGY AND MEDICINE 57 (1984), 627-641

Avian and Mammalian Hosts for Spirochete-Infected Ticks and Insects in a Lyme Disease Focus in Connecticut

JOHN F. ANDERSON, Ph.D., AND LOUIS A. MAGNARELLI, Ph.D.

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, Connecticut

Received November 16, 1983

Spirochetes and their vectors and reservoirs were studied in a Lyme disease focus in East Haddam, Connecticut, from mid-May through September 1983. *Ixodes dammini* subadults were comparable in number on white-footed mice (*Peromyscus leucopus*) ($\bar{X}$ = 2.9 ± 3.6 SD) to those on 27 different species of birds ($\bar{X}$ = 2.3 ± 4.2 SD) representing 11 families within the order Passeriformes. Less commonly found ticks on birds ($\bar{X} \leq 0.1$) were immature *Ixodes dentatus* and *Haemaphysalis leporispalustris*. Although spirochete-infected *I. dammini* larvae and nymphs were taken off eight and nine different species of birds, respectively, significantly fewer positive larvae were removed from birds than from white-footed mice.

Spirochetes were detected in the midguts of *I. dammini*, *Dermacentor variabilis*, and *H. leporispalustris* and two species of insects (*Cuterebra fontinella* and *Orchopeas leucopus*). Possibly, arthropods other than *I. dammini* vector these spirochetes in northeastern United States.

Spirochetes grew in a cell-free medium inoculated with bloods from four white-footed mice, one woodland jumping mouse (*Napaeozapus insignis*), one northern mockingbird (*Mimus polyglottos*), one gray catbird (*Dumetella carolinensis*), two prairie warblers (*Dendroica discolor*), one orchard oriole (*Icterus spurius*), one common yellowthroat (*Geothlypis trichas*), and one American robin (*Turdus migratorius*). We suggest that avian hosts, like mammals, develop spirochetemias of the causative agent of Lyme disease. Erythematous tissues from a white-footed mouse were infected with spirochetes.

The zoonosis known as Lyme disease [1] is caused by a spirochete that is vectored by ixodid ticks [2]. *Ixodes dammini,* a species with a wide host range including small-, medium-, and large-sized mammals and birds [3-9], is considered the principal vector in northeastern United States. The etiologic agent has been isolated from juvenile and adult *I. dammini,* humans, and wild mammals [2,10-13].

This disease or related disorders have been reported from three continents (North America [1,10], Australia [14], and Europe [15,16]). Spirochetes with similar surface antigens to those grown from *I. dammini,* humans, and wild mammals in northeastern United States have been isolated from *I. ricinus* collected in Switzerland [17,18] and are present in *I. pacificus* in western United States [2]. There are several epidemiologic questions concerning dispersal, reservoirs, and vectors of these apparent globally distributed bacteria. We attempted to answer some of these questions by examining birds for ticks, and by testing ticks, parasitic insects, and their avian and mammalian hosts for spirochetes in a Lyme disease focus at East Haddam, Connecticut. We report here the recovery of juvenile *I. dammini* from 27

Address reprint requests to: John F. Anderson, Ph.D., Dept. of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504

species of birds, the prevalence of ticks on birds and white-footed mice (*Peromyscus leucopus*), and the presence of spirochete-infected larval and nymphal ticks on birds and white-footed mice. We also describe the finding of spirochetes in erythematous tissues of a white-footed mouse, the growth of spirochetes in fortified Kelly's medium inoculated with blood drawn from birds and small mammals, and the occurrence of spirochetes in a flea and a bot fly larva.

MATERIALS AND METHODS

Arthropod Collections

Ticks and insects were removed from vertebrate hosts from mid-May through September 1983. Mammals were captured and ticks removed by methods described previously [6]. Birds were captured in Japanese mist nets. The head and neck of each bird were examined at 10–20 × magnification. Feathers on the neck and head were "combed," and all ticks were removed and placed into labeled vials containing a blade of grass. Fleas and bot fly larvae were removed from white-footed mice.

The Lyme disease focus selected for study was previously designated as East Haddam site number 2 and is a 105-hectare farm comprising hay fields, pasture, hedgerows, and woodlands [12]. Mammals were caught in live traps along streams, hedgerows, stone walls, and woodlands and were released at their sites of capture after ticks were removed. Blood samples were drawn and individuals were identified with an ear tag. Mist nets were placed between and adjacent to hedgerows, across streams, in open or partially opened hay fields or pasture, and in woodlands. Birds were released after they had been examined for ticks and blood had been drawn.

Attempts to Isolate Spirochetes

Sterile whole or heparinized blood was drawn from the hearts of small mammals and from the jugular veins of birds. Each host was initially cleansed in the area of needle insertion with Betadine Surgical Scrub®. Varying amounts of blood from one drop (1 drop = 0.01 ml) to 0.2 ml were inoculated into 7–10 ml of fortified Kelly's medium [2,19,20]. Inoculated media were kept at 33°C and examined by darkfield microscopy for the presence of spirochetes at periodic intervals for four to six weeks. Attempts to isolate spirochetes from ticks and fleas were made from surface-sterilized arthropods as described previously [12].

Isolates from nymphal ticks were cross-tested by using indirect immunofluorescence with CT isolate number 2591 that had been grown from the blood of a white-footed mouse [12]. Test antigens were spirochetes grown in fortified Kelly's medium. Eight ml of infected culture medium were centrifuged at 35,000 *g* for 30 minutes and all but the bottom 0.5 ml of supernatant were discarded. Concentrated spirochetes were mixed 1:1 with 5 percent yolk sac diluted in phosphate-buffered saline containing 0.01 percent sodium azide or aqueous thimersol. Antisera to isolates were prepared in weanling Swiss mice inoculated on days 0 and 7 and exsanguinated on days 14–18.

Examination of Tick Midguts and Erythematous Skin Tissues for Spirochetes

Midgut tissues were examined for spirochetes by direct fluorescent antibody staining (FA). Each live tick or insect was dissected in phosphate-buffered saline (pH 7.2), and the midgut tissue was smeared on a glass slide. Tissues were air-dried for 24 to 48 hours at room temperature before being fixed in acetone and overlaid with high-titered fluorescein isothiocyanate-labeled (FITC) rabbit antibody against spiro-

chetes isolated from *I. dammini.*[1] Stained slides were mounted with buffered glycerol and examined for spirochetes with a Zeiss fluorescence microscope.

Erythematous skin tissues from the ears of two white-footed mice were immediately frozen in Tissue-tek® II OCT compound. Preparations were sectioned in a Model CTD International Harris cryostat at 6–8μ. Sections were applied to glass slides and allowed to dry for 24–48 hours at room temperature (21 ± 3°C). They were then fixed in acetone, stained by direct FA, and examined for spirochetes as described above.

Significant differences in means were determined by Student *t*-test for unpaired data when sample variances were statistically homogeneous by F-tests [21]. Means were compared by Student *t*-test for non-homogeneous variances. Chi-square test was used to determine levels of significance for prevalence of spirochete-infected ticks on various hosts.

RESULTS

Three hundred and twenty-three birds representing 49 species were collected in mist nets from May through September 1983 in East Haddam. Fifty-five percent of these species (n = 27), including 11 families of Passeriformes, were parasitized by juvenile *I. dammini* (Table 1). Mean numbers of ticks on birds collected ten or more times ranged as high as 3.5 larvae per swamp sparrow and 4.2 nymphs per American robin. Other species that averaged one or more larvae per bird or had at least one larva when only one bird was examined included blue jay, house wren, gray catbird, American robin, Swainson's thrush, pine warbler, northern waterthrush, common yellowthroat, yellow-breasted chat, brown-headed cowbird, northern cardinal, rufous-sided towhee, chipping sparrow, and field sparrow. An average of 0.5 to 4.2 nymphs per bird was recorded for blue jay, black-capped chickadee, white-breasted nuthatch, American robin, hermit thrush, Louisiana waterthrush, common yellowthroat, common grackle, brown-headed cowbird, northern cardinal, rose-breasted grosbeak, purple finch, chipping sparrow, field sparrow, and swamp sparrow. As many as 21 larvae were removed from a gray catbird and a swamp sparrow; an American robin carried 19 nymphs at the time of capture.

Less commonly found ticks were immature *Ixodes dentatus* and *Haemaphysalis leporispalustris.* Seven species were parasitized by *I. dentatus,* and five species carried *H. leporispalustris* (Table 2). With the exception of the red-eyed vireo (*Vireo olivaceus*) and Nashville warbler (*Vermivora ruficapilla*), all species were also parasitized by *I. dammini.*

Subadult ticks were removed from birds and white-footed mice throughout the sampling period (Table 3). Larval *I. dammini* were most prevalent from the latter part of July through September; nymphs were most numerous during June. *Dermacentor variabilis* larvae parasitized white-footed mice from early April through mid-July, and nymphs were active from early June through July. *Ixodes dentatus* larvae were prevalent on birds during September when the study was terminated.

Short-tailed shrew (*Blarina brevicauda*), raccoon (*Procyon lotor*), and woodland jumping mouse (*Napaeozapus insignis*) were also collected. Of the 13 shrews examined, eight had larval *I. dammini.* A total of 147 larvae were removed, but 117 came from one host. Two larvae parasitized one of two woodland jumping mice,

[1]The FITC-labeled rabbit antibody against spirochetes isolated from *I. dammini* was provided by Dr. Allan C. Steere.

TABLE 1
Birds Infested with Immature *Ixodes dammini* in East Haddam, Connecticut, May–September 1983

Common Name	Scientific Name	No. Examined	No. Birds Infested (%)	Mean No. Ticks per Bird ± S.D.		Maximum No. Ticks Collected Off a Single Bird	
				Larvae	Nymphs	Larvae	Nymphs
Tyrannidae							
Eastern phoebe	*Sayornis phoebe*	10	1 (10)	0	0.1 ± 0.3	0	1
Corvidae							
Blue jay	*Cyanocitta cristata*	1	1 (100)	–	–	1	1
Paridae							
Black-capped chickadee	*Parus atricapillus*	26	7 (27)	0.2 ± 0.4	0.6 ± 1.8	1	7
Sittidae							
White-breasted nuthatch	*Sitta carolinensis*	2	2 (100)	0.5 ± 0.7	0.5 ± 0.7	1	1
Troglodytidae							
House wren	*Troglodytes aedon*	3	2 (67)	2.0 ± 1.7	0	3	0
Mimidae							
Gray catbird	*Dumetella carolinensis*	84	45 (54)	1.3 ± 3.3	0.3 ± 0.6	21	3
Turdidae							
American robin	*Turdus migratorius*	11	7 (64)	1.1 ± 3.3	4.2 ± 6.2	11	19
Swainson's thrush	*Catharus ustulatus*	6	6 (100)	6.8 ± 7.4	0.2 ± 0.4	18	1
Hermit thrush	*Catharus guttatus*	1	1 (100)	–	–	0	6

Vireonidae							
White-eyed vireo	*Vireo griseus*	12	1 (8)	0.2 ± 0.6	0	2	0
Parulidae							
Blue-winged warbler	*Vermivora pinus*	5	3 (60)	0.4 ± 0.5	0.4 ± 0.9	1	2
Pine warbler	*Dendroica pinus*	1	1 (100)	–	–	2	0
Northern waterthrush	*Seiurus noveboracensis*	1	1 (100)	–	–	4	0
Louisiana waterthrush	*Seiurus motacilla*	2	1 (50)	0	1.0 ± 1.4	0	2
Common yellowthroat	*Geothlypis trichas*	27	17 (63)	2.6 ± 5.1	0.5 ± 0.6	18	2
Yellow-breasted chat	*Icteria virens*	1	1 (100)	–	–	1	0
Icteridae							
Red-winged blackbird	*Agelaius phoeniceus*	4	1 (25)	0.5 ± 1.0	0.3 ± 0.5	2	1
Common grackle	*Quiscalus quiscula*	1	1 (100)	–	–	0	3
Brown-headed cowbird	*Molothrus ater*	6	5 (83)	2.5 ± 2.1	1.0 ± 2.5	5	6
Fringillidae							
Northern cardinal	*Cardinalis cardinalis*	2	2 (100)	2.0 ± 2.8	1.0 ± 1.4	4	2
Rose-breasted grosbeak	*Pheucticus ludovicianus*	3	2 (67)	0	2.0 ± 2.0	0	4
Purple finch	*Carpodacus purpureus*	3	1 (33)	0	0.7 ± 1.2	0	2
Rufous-sided towhee	*Pipilo erythrophthalmus*	1	1 (100)	–	–	4	0
Chipping sparrow	*Spizella passerina*	1	1 (100)	–	–	2	5
Field sparrow	*Spizella pusilla*	3	3 (100)	1.7 ± 2.1	1.3 ± 1.5	4	3
White-throated sparrow	*Zonotrichia albicollis*	4	2 (50)	0.5 ± 0.6	0	1	0
Swamp sparrow	*Melospiza georgiana*	55	45 (82)	3.5 ± 5.3	0.5 ± 1.2	21	6
Total		276	161				

TABLE 2
Birds Infested with Immature *Ixodes dentatus* and *Haemaphysalis leporispalustris*, East Haddam, Connecticut, 1983

Common Name	No. Birds Examined	No. Birds Infested with		$\bar{X}$ No. *I. dentatus* per Bird		$\bar{X}$ No. *H. leporispalustris* per Bird	
		I. dentatus	*H. leporis-palustris*	Larvae	Nymphs	Larvae	Nymphs
Blue jay	1	1	0	3	0	0	0
Northern cardinal	2	0	1	0	0	0	0.5 ± 0.7
Gray catbird	84	8	1	0.3 ± 1.3	0	0.1 ± 0.1	0
Black-capped chickadee	26	1	0	0.1 ± 0.2	0	0	0
Common grackle	1	1	0	0	1	0	0
House wren	3	1	0	0.7 ± 1.1	0	0	0
Nashville warbler	1	0	1	0	0	1	0
Red-eyed vireo	1	0	1	0	0	1	0
American robin	11	1	0	0.1 ± 0.3	0.3 ± 0.9	0	0
Swainson's thrush	6	0	1	0	0	0	0.2 ± 0.4
White-throated sparrow	4	1	0	1.0 ± 2.0	0	0	0
Total	140	14	5				

TABLE 3
Mean Number of Juvenile Ticks per White-Footed Mouse and per Bird[a] Captured in East Haddam, Connecticut, April–September 1983

Date	No. Mice Examined	$\bar{X}$ ± SD/Mouse				No. Birds Examined	$\bar{X}$ ± SD/Bird					
		I. dammini		*D. variabilis*			*I. dammini*		*I. dentatus*		*H. leporispalustris*	
		Larvae	Nymphs	Larvae	Nymphs		Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs
April 1–15	6	0	0	1.3 ± 2.2	0	0						
April 16–30	20	0.7 ± 1.3	0.2 ± 0.4	3.1 ± 3.6	0	0						
May 1–15	11	0.8 ± 1.1	0.4 ± 0.6	4.3 ± 4.7	0	0						
May 16–31	13	0.6 ± 0.9	0.8 ± 1.0	2.0 ± 1.7	0	16	0.1 ± 0.3	0.4 ± 1.1	0.1 ± 0.3	0.2 ± 0.8	0.1 ± 0.3	0
June 1–15	15	0.5 ± 1.0	3.4 ± 3.2	1.5 ± 2.0	0.2 ± 0.8	28	0	0.5 ± 1.3	0	<0.1 ± 0.2	0	0
June 16–30	23	1.3 ± 2.8	0.7 ± 1.7	0.4 ± 1.0	0.2 ± 0.7	48	0.3 ± 1.6	1.3 ± 3.5	0	0	0	0
July 1–15	20	1.0 ± 1.9	0.6 ± 1.0	0.2 ± 0.6	0.2 ± 0.5	25	0.1 ± 0.4	1.7 ± 2.1	0	0	0	0
July 16–31	15	2.9 ± 3.0	0.2 ± 0.4	0	0.1 ± 0.5	52	1.7 ± 3.7	0.3 ± 0.7	0	0	0	0
Aug. 1–15	5	2.2 ± 2.9	0	0	0	64	3.9 ± 5.6	0.3 ± 0.6	0	0	0	0
Aug. 16–30	12	5.2 ± 5.6	0	0	0	0						
Sept. 1–15	13	5.5 ± 4.5	0	0	0	38	1.8 ± 3.2	0.2 ± 0.4	<0.1 ± 0.2	0	<0.1 ± 0.2	<0.1 ± 0.2
Sept. 16–30	27	2.1 ± 3.5	0.2 ± 0.5	<0.1 ± 0.2	0	52	1.1 ± 3.0	0.1 ± 0.3	0.7 ± 1.7	0	<0.1 ± 0.1	<0.1 ± 0.1
Total	188					323						

[a]Includes all birds captured

and one larva and two nymphs were observed on three raccoons. Thirty-three adult *D. variabilis* and three *I. texanus* were also removed from raccoons.

Mean numbers of *I. dammini* subadults ($\bar{X}$ = 2.9 ± 3.6 SD) parasitizing white-footed mice were not significantly different from subadult ($\bar{X}$ = 2.3 ± 4.2 SD) numbers recorded for all species of infested birds listed in Table 1. Among birds frequently captured ($n \geq 20$) and carrying an average of > 1.5 ticks/host, mean larval and nymphal numbers on swamp sparrows and common yellowthroats were similar to those on white-footed mice (Table 4). Significantly fewer nymphs and total juvenile ticks parasitized gray catbirds than infested white-footed mice. Among the three species of birds, significantly greater mean numbers of larvae and total subadults fed on swamp sparrows than gray catbirds.

Larval and nymphal *I. dammini* collected from mammals and birds were infected with spirochetes (Table 5). Of the 181 larvae examined from 51 white-footed mice, 46 (25.4 percent) were positive. Twenty different mice carried infected larvae. Similarly, 18 of 63 nymphs (28.6 percent) removed from 32 mice harbored spirochetes. Thirty-one of 35 larvae taken off one short-tailed shrew were positive; nine other larvae obtained from five other shrews were negative.

Spirochete-infected *I. dammini* larvae were taken off the following species of birds: gray catbird, brown-headed cowbird, field sparrow, house wren, pine warbler, American robin, swamp sparrow, and common yellowthroat. Infected larvae were removed from 13 swamp sparrows, three gray catbirds, three common yellowthroats, and two brown-headed cowbirds. Single individuals of the four other species yielded positive ticks. Infected *I. dammini* nymphs were observed feeding on nine species of birds (blue-winged warbler, gray catbird, field sparrow, common grackle, hermit thrush, Louisiana waterthrush, American robin, swamp sparrow, and common yellowthroat).

Spirochete-infected *I. dammini* larvae were significantly more abundant on white-footed mice than on the eight species of birds carrying infected larvae (X^2 = 7.14, 1 df, $p < 0.01$) and specifically on gray catbirds (X^2 = 6.63, 1 df, $p < 0.01$). Infection rates were statistically homogeneous with those recorded for larval ticks parasitizing swamp sparrows (X^2 = 1.36, 1 df, $p < 0.05$) and common yellowthroats (X^2 = 2.68, 1 df, $p < 0.05$).

Erythemas were noted in the ears of five white-footed mice. There were no ticks attached at these sites at the time of capture. Two white-footed mice had erythemas measuring approximately 5 mm × 2 mm, 1 mm × 1 mm, and 3 mm × 1.5 mm. As many as 12 spirochetes were observed by direct FA in a section of the 3 mm × 1.5

TABLE 4

Mean Numbers of Larval, Nymphal, and All Juvenile *Ixodes dammini* on White-Footed Mice and Three Species of Birds, May 16–Aug. 15 and Sept. 1–Sept. 30, 1983, East Haddam, Connecticut

Host	No. Hosts Examined	Mean Ticks/Host		
		Larvae	Nymphs	Larvae and Nymphs
White-footed mouse	122	2.1 ± 3.3 a c[a]	0.8 ± 1.8 a	2.9 ± 3.6 a
Swamp sparrow	55	3.5 ± 5.3 a	0.5 ± 1.2 a ç	4.1 ± 5.4 a
Common yellowthroat	27	2.6 ± 5.1 a c	0.4 ± 0.6 a c	3.0 ± 5.3 a c
Gray catbird	84	1.3 ± 3.3 b c	0.3 ± 0.6 b c	1.6 ± 3.3 b c

[a] Values within columns followed by the same letter are not significantly different at 5 percent level, *t*- and *t'* tests.

TABLE 5
Prevalence of Spirochete-Infected Juvenile *I. dammini* Parasitizing Birds and Mammals, East Haddam, Connecticut, 1983

Host	Larvae			Nymphs		
	No. Hosts	No. Examined	No. Positive (%)	No. Hosts	No. Examined	No. Positive (%)
White-footed mouse	51	181	46 (25)	32	63	18 (29)
Short-tailed shrew	6	44	31 (70)			
Raccoon				2	2	0
Blue Jay				1	1	0
Black-capped chickadee	3	3	0	3	6	0
White-breasted nuthatch				1	1	
House wren	1	2	1 (50)			
Gray catbird	22	71	5 (7)	18	21	4 (19)
American robin	1	4	1 (25)	7	41	12 (29)
Swainson's thrush	3	34	0			
Hermit thrush				1	4	2 (50)
Blue-winged warbler	2	2	0	1	1	1 (100)
Pine warbler	1	3	2 (67)			
Northern waterthrush	1	3	0			
Louisiana waterthrush				1	2	1 (50)
Common yellowthroat	10	47	8 (17)	7	7	3 (43)
Yellow-breasted chat	1	1	0			
Red-winged blackbird	1	4	0			
Common grackle				1	3	1 (33)
Brown-headed cowbird	3	10	3 (30)	1	5	0
Northern cardinal	1	2	0	1	1	0
Rose-breasted grosbeak				1	3	0
Purple finch				1	1	0
Rufous-sided towhee	1	1	0			
Field sparrow	2	2	1 (50)	1	1	1 (100)
Swamp sparrow	35	163	31 (19)	13	26	5 (19)
Total	145	577	129	93	189	48

mm erythematous tissue. No spirochetes were seen in fluorescein-stained sections of the second mouse. Spirochetes did not grow in Kelly's medium following inoculation of blood from either mouse.

Tissues of midguts from *D. variabilis, H. leporispalustris, I. dentatus, I. texanus,* a bot fly (*Cuterebra fontinella*), and a flea (*Orchopeas leucopus*) were examined for spirochetes by direct FA and by inoculation into culture media (Table 6). Eight of 141 *D. variabilis* larvae feeding on white-footed mice had spirochetes. These bacteria were cultured from five of 54 ticks. A partially engorged nymph also contained spirochetes. Two of the mice with positive *D. variabilis* larvae were recaptured 21 days later with attached individuals harboring spirochetes. Similar organisms were detected by direct FA in the midgut of a *H. leporispalustris* nymph feeding on a Swainson's thrush, but we did not culture these bacteria from duplicate tissues of the same tick. No spirochetes were found in *I. dentatus* or *I. texanus,* but these agents were present in the midgut of a *C. fontinella* larva that emerged from a white-footed mouse. Spirochetes grew in Kelly's medium following inoculation of midgut tissues of two *O. leucopus.* Cultures were contaminated and were not examined further.

TABLE 6
Prevalence of Spirochetes in *Dermacentor variabilis, Haemaphysalis leporispalustris, Ixodes dentatus, Ixodes texanus, Cuterebra fontinella,* and *Orchopeas leucopus,* East Haddam, Connecticut, 1983

Arthropod Species	Host or Questing	Stage	No. Positive/No. Examined	
			DFA	Culture
D. variabilis	Questing	Larva	0/1	
	Questing	Female	0/16	0/2[a]
	Questing	Male	0/10	0/1
	Raccoon	Female	0/10	0/3
	Raccoon	Male	0/11	
	White-footed mouse	Larva	8/141	5/54
	White-footed mouse	Nymph	1/11	0/1
H. leporispalustris	Nashville warbler	Larva	0/1	
	Gray catbird	Larva	0/1	
	Swainson's thrush	Nymph	1/1	
	Northern cardinal	Nymph		0/1
I. dentatus	Birds[b]	Larva	0/9	0/18
		Nymph	0/4	0/4
I. texanus	Raccoon	Female	0/3	0/2
C. fontinella	White-footed mouse	Larva	1/5	
O. leucopus	White-footed mouse	Adult	0/10	2/19

[a]Midguts of nymphal and adult ticks were sometimes examined for spirochetes by DFA and by placement into culture. The denominator shown in the culture column for nymphs and adult ticks is included in the denominator of the DFA column.
[b]American robin, common grackle, gray catbird, house wren, white-throated sparrow, blue jay

Attempts to isolate spirochetes in fortified Kelly's medium were also made from the bloods of 310 birds, 176 white-footed mice, two woodland jumping mice, 11 short-tailed shrews, and three raccoons, as well as midgut tissues of 129 *I. dammini* larvae and 22 nymphs. Spirochetes grew in media inoculated with blood from four white-footed mice, one woodland jumping mouse, one northern mockingbird (*Mimus polyglottos*), one gray catbird, two prairie warblers (*Dendroica discolor*), one orchard oriole (*Icterus spurius*), one common yellowthroat, and one American robin. Additionally, isolations were made from midgut tissues of *I. dammini* nymphs that had been feeding on white-footed mice ($n = 2$), common yellowthroat (Connecticut #4018), common grackle (Connecticut #4085), blue-winged warbler (Connecticut #4097), and hermit thrush ($n = 2$) (Connecticut #4288 and #4290). With the exception of the five isolates made from nymphs feeding on birds, all spirochetes died in culture and were not tested further. In reciprocal cross-tests, antigens and Swiss mouse antisera from *I. dammini* nymphs feeding on a common grackle, blue-winged warbler, hermit thrush, and a common yellowthroat reacted similarly to those of Connecticut isolate #2591 from a white-footed mouse.

DISCUSSION

Ixodes dammini parasitizes many warm-blooded animals. At least 29 species of mammals are known to serve as hosts [3–8]. Previous reports [4–6,8,9] coupled with our identifications of bird hosts bring the total to 38. With the exception of the common bobwhite (*Colinus virginianus*), order Galliformes, all infested bird species are classified in the order Passeriformes. These records probably reflect more the trapping methods rather than host preference.

As with mammals, not all bird species are equally infested. Seemingly, ground-inhabiting species are most likely to encounter ticks. Among these, however, certain species have larger numbers of ticks. Swamp sparrows, for example, have proportionately more ticks than gray catbirds. Behavioral differences that bring one species into more frequent contact with ticks may account for this diversity.

The importance of birds as maintenance and dispersal hosts for *I. dammini* has been discussed [4,8,9,22]. Clearly, our studies show that some species of birds are parasitized as frequently by larvae and nymphs as are white-footed mice, a significant host for juvenile *I. dammini* [3–8]. Like many small rodents, nesting (i.e., common yellowthroat) or year-round resident (i.e., swamp sparrow) birds are notable hosts for maintaining tick populations.

Migratory birds may transport ticks over considerable distances [23]. In addition to other *Ixodes* species, *I. dammini* would likewise appear to be passively moved short or long distances. Of the 27 infested species, Swainson's thrush, hermit thrush, white-throated sparrow, and yellow-breasted chat are transients that are not known to nest in the East Haddam area.[2] Ten other species (eastern phoebe, house wren, gray catbird, white-eyed vireo, pine warbler, blue-winged warbler, common yellowthroat, northern waterthrush, Louisiana waterthrush, and rose-breasted grosbeak) nest in the area,[2] but migrate to the South during winter [24]. Even among birds considered to be year-round residents [24], major portions of populations of such species as American robin and common grackle migrate southward in the fall. Thus, birds also appear to be vital incidental hosts serving to disperse ticks into new areas.

A composite diagram (Fig. 1), patterned after an idea of Audy [25], shows the life stages of *I. dammini,* the relationship of this tick to its vertebrate hosts [3–9,26,27], and the tick's natural means of dispersal. Mammals and birds are either maintenance or incidental hosts; the former represent animals (i.e., mice, birds, chipmunks, white-tailed deer) that regularly and dependably serve as sources of blood for the motile ticks near or in habitats suitable for survival. Incidental hosts are those which chiefly serve to disperse the parasite (i.e., birds, transient hunting dogs) or those such as man that are fed upon occasionally but, compared to other hosts, do not significantly enhance tick survival.

Juvenile *I. dammini* infected with spirochetes were removed from white-footed mice, a short-tailed shrew, and ground-inhabiting birds. Larvae became infected either transovarially or by ingestion of spirochetes. Although transovarial transmission has been demonstrated in *I. ricinus* [18], it has not yet been shown to occur in *I. dammini,* though it cannot be ruled out. Nymphs may vector spirochetes to both mammals and birds. Our data suggest the possibility of subadults acquiring spirochetes from both of these warm-blooded vertebrates.

Geographic clustering of children with arthritis eventually led to the recognition of the new disease entity known as Lyme disease [1]. The relatively large number of larval *I. dammini* found on a single short-tailed shrew ($n = 117$) and the extremely high rate of infection among those ticks (89 percent) illustrate how a single small animal may be responsible for the aggregation of infected nymphs. These positive-tick assemblages may partly explain the non-random distribution of human cases within broad geographic foci.

Juvenile forms of three other species of ticks shared some of the same hosts with *I. dammini. Dermacentor variabilis* larvae were prevalent on white-footed mice.

[2]Clay Taylor, Region 5 Coordinator of the Connecticut Breeding Bird Atlas Project, provided this information.

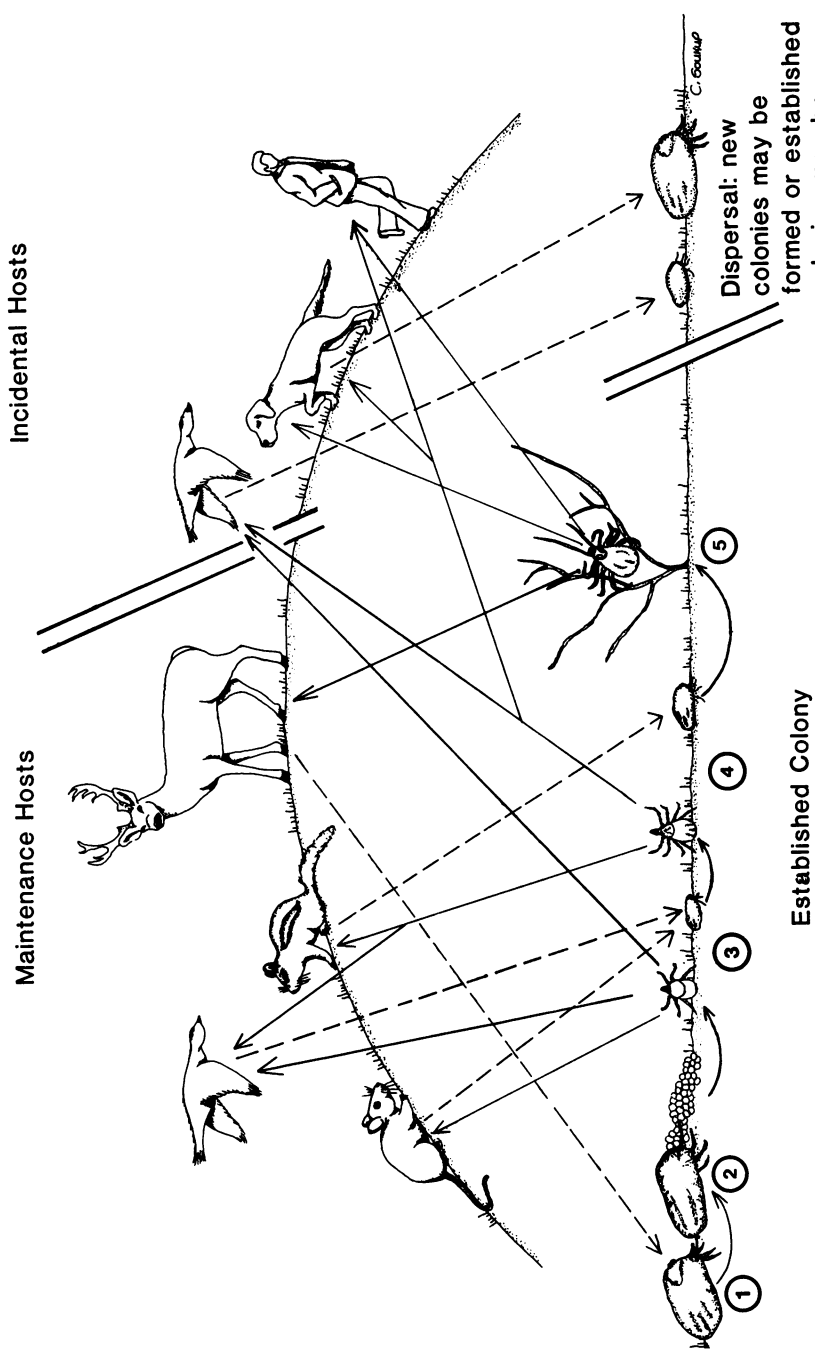

FIG. 1. Composite diagram of the life cycle of *Ixodes dammini*. **1**—engorged female; **2**—adult female laying eggs; **3**—questing and engorged larva; **4**—questing and engorged nymph; **5**—questing adult. ——➤ questing tick successfully finds host; - - - ➤ tick engorges and drops from host; ⤻ tick develops to next stage.

Larval *I. dentatus* and larval and nymphal *H. leporispalustris* parasitized birds as noted previously [28]. Thus, pathogens, such as spirochetes, may be transferred from one tick species to another via tissues of a mutual host.

Spirochetes were observed in midguts of feeding subadult *D. variabilis* and *H. leporispalustris,* two species not heretofore recognized to harbor spirochetes. Infected ticks may have obtained their spirochetes directly from their mammalian or avian hosts. As yet, there is no evidence that this spirochete is trans-stadially or transovarially transmitted in ticks other than those within the *I. ricinus* complex. In fact, adult *D. variabilis* were negative for spirochetes here as in an earlier study [12]. The recovery of positive larval *D. variabilis* from the same individual hosts 21 days apart indicates either that mice remained spirochetemic or that they repeatedly encountered infected, questing larvae. Further studies are needed to clarify the maintenance and transmission of spirochetes in these tick species.

The observation of spirochetes in a bot fly larva and the culturing of spirochetes from midguts of adult fleas are the first reports of these organisms in insects. Most likely, the cuterebrid larva ingested spirochetes from host tissues. *Orchopeas leucopus,* a common flea on white-footed mice [29], may also have acquired these agents directly from its host, but it is unclear whether these arthropods are vectors. Since erythema chronicum migrans has been linked to insect bites [30–32] and since most people diagnosed with Lyme disease do not remember being bitten by a tick [12,33–35], arthropods other than ticks may possibly vector these bacteria. The possible importance of hematophagous insects in the epidemiology of Lyme disease needs consideration.

Erythema chronicum migrans, a unique skin lesion, is diagnostic of Lyme disease [1,10,33]. Similar lesions described as multiple, enlarging annular or irregularly-shaped erythemas were observed in laboratory rabbits fed upon by infected *I. dammini* or *I. ricinus* ticks [2,18]. Our finding of erythematous skin tissues infected with spirochetes in a white-footed mouse suggests that wild mammals may have lesions similar to those described in humans and rabbits.

Lyme disease-causing spirochetes with apparently similar antigenic determinants [36] have been isolated from humans [10,11], white-footed mice [12,13,37], a meadow vole (*Microtus pennsylvanicus*) [13], white-tailed deer (*Odocoileus virginianus*) [13], and a raccoon [12]. Antibodies to these spirochetes have been detected in domestic and wild mammals [37–39]. In this study, spirochetes were recovered from the blood of four white-footed mice, one woodland jumping mouse, one northern mockingbird, one gray catbird, two prairie warblers, one orchard oriole, one common yellowthroat, and one American robin. The culturing of spirochetes from birds and the presence of these organisms in *I. dammini* feeding on these hosts strongly suggest that avians, like mammals, are spirochetemic. Therefore, birds may be important reservoirs for Lyme disease-causing spirochetes and may transport these bacteria and ticks into new geographic areas.

ACKNOWLEDGEMENTS

We thank Carol Lemmon, Jim Brassard, Deborah Shea, and RoseAnn LaBarre for their technical assistance. C. Soukup and P.M. Trzcinski prepared the figure.

REFERENCES

1. Steere AC, Malawista SE, Snydman DR, et al: Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 20:7–17, 1977

2. Burgdorfer W, Barbour AG, Hayes SF, et al: Lyme disease—a tick-borne spirochetosis? Science 216:1317–1319, 1982
3. Wallis RC, Brown SE, Kloter KO, et al: Erythema chronicum migrans and Lyme arthritis: Field study of ticks. Am J Epidemiol 108:322–327, 1978
4. Spielman AC, Clifford CM, Piesman J, et al: Human babesiosis on Nantucket Island, USA: description of the vector, *Ixodes* (*Ixodes*) *dammini,* n. sp. (Acarina: Ixodidae). J Med Entomol 15:218–234, 1979
5. Carey AB, Krinsky WL, Main AJ: *Ixodes dammini* (Acari: Ixodidae) and associated ixodid ticks in south-central Connecticut, USA. J Med Entomol 17:89–99, 1980
6. Anderson JF, Magnarelli LA: Vertebrate host relationships and distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J Med Entomol 17:314–323, 1980
7. Carey MG, Carey AB, Main AJ, et al: *Ixodes dammini* (Acari: Ixodidae) in forests in Connecticut. J Med Entomol 18:175–176, 1981
8. Main AJ, Carey AB, Carey MG, et al: Immature *Ixodes dammini* (Acari: Ixodidae) on small animals in Connecticut, USA. J Med Entomol 19:655–664, 1982
9. Main AJ, Anderson KS: Avian ectoparasites at Manomet Bird Observatory. M B O Res Rep No 2:7, 1971
10. Steere AC, Grodzicki RL, Kornblatt AN, et al: The spirochetal etiology of Lyme disease. New Eng J Med 308:733–740, 1983
11. Benach JL, Bosler EM, Hanrahan JP, et al: Spirochetes isolated from the blood of two patients with Lyme disease. New Eng J Med 308:740–742, 1983
12. Anderson JF, Magnarelli LA, Burgdorfer W, et al: Spirochefes in *Ixodes dammini* and mammals from Connecticut. Am J Trop Med Hyg 32:818–824, 1983
13. Bosler EM, Coleman JL, Benach JL, et al: Natural distribution of the *Ixodes dammini* spirochete. Science 220:321–322, 1983
14. Stewart A, Glass J, Patel A, et al: Lyme arthritis in the Hunter Valley. Med J Aust 1:139, 1982
15. Ackermann R, Runne U, Klenk W, et al: Erythema Chronicum Migrans mit Arthritis. Dtsch Med Wochenschr 105:1779–1781, 1980
16. Gerster JC, Guggi S, Perroud H, et al: Lyme arthritis appearing outside the United States: a case report from Switzerland. Br Med J 283:951–952, 1981
17. Barbour AG, Burgdorfer W, Hayes SF, et al: Isolation of a cultivable spirochete from *Ixodes ricinus* ticks of Switzerland. Curr Microbiol 8:123–126, 1983
18. Burgdorfer W, Barbour AG, Hayes SF, et al: Erythema chronicum migrans—a tickborne spirochetosis. Acta Tropica 40:79–83, 1983
19. Kelly R: Cultivation of *Borrelia hermsi.* Science 173:443–444, 1971
20. Stoenner HG, Dodd T, Larsen C: Antigenic variation of *Borrelia hermsii.* J Exp Med 156:1297–1311, 1982
21. Ostle B: Statistics in Research, Basic Concepts and Techniques for Research Workers, Second Edition. Ames, Iowa, The Iowa State University Press, 1963, 585 pp
22. McEnroe WD: Human babesiosis. Science 195:506–507, 1977
23. Hoogstraal H, Kaiser MN, Traylor MA, et al: Ticks (Ixodidae) on birds migrating from Europe and Asia to Africa, 1959–61. Bull WHO 28:235–262, 1963
24. Peterson RT: A Field Guide to the Birds, A Completely New Guide to All the Birds of Eastern and Central North America. Boston, Houghton Mifflin Company, 1980, 384 pp
25. Audy JR: The ecology of scrub typhus. In Studies in Disease Ecology. Edited by JM May. New York, Hafner Publishing Company Incorporated, 1961, pp 389–432
26. Piesman J, Spielman A, Etkind P, et al: Role of deer in the epizootiology of *Babesia microti* in Massachusetts, USA. J Med Entomol 15:537–540, 1978
27. Main AJ, Sprance HE, Kloter KO, et al.: *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer (*Odocoileus virginianus*) in Connecticut. J Med Entomol 18:487–492, 1981
28. Sonenshine DE, Clifford CM: Contrasting incidence of Rocky Mountain spotted fever in ticks infesting wild birds in eastern U.S. Piedmont and coastal areas, with notes on the ecology of these ticks. J Med Entomol 10:497–502, 1973
29. Main AJ: Fleas (Siphonaptera) on small mammals in Connecticut, USA. J Med Entomol 20:33–39, 1983
30. Hellerström S: Erythema chronicum migrans Afzelius with meningitis. South Med J 43:330–335, 1950
31. Sonck CE: Erythema Chronicum Migrans im Gesicht bei Drei Kindern. Acta Derm-Venereol 34:482–486, 1954

32. Hard S: Erythema chronicum migrans (Afzelii) associated with mosquito bite. Acta Derm-Venereol 46:473–476, 1966
33. Steere AC, Barthenhagen NH, Craft JE, et al: The early clinical manifestations of Lyme disease. Ann Intern Med 99:76–82, 1983
34. Steere AC, Broderick TF, Malawista SE: Erythema chronicum migrans and Lyme arthritis: Epidemiologic evidence for a tick vector. Am J Epidemiol 108:312–321, 1978
35. Magnarelli LA, Anderson JF, Chappell WA: Geographic distribution of humans, raccoons, and white-footed mice with antibodies to Lyme disease spirochetes in Connecticut. Yale J Biol Med 57:619–626, 1984
36. Barbour AG, Tessier SL, Todd WJ: Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect Immun 41:795–804, 1983
37. Anderson JF, Magnarelli LA: Spirochetes in *Ixodes dammini* and *Babesia microti* on Prudence Island, Rhode Island. J Infect Dis 148:1124, 1983
38. Magnarelli LA, Anderson JF, Burgdorfer W, et al: Parasitism by *Ixodes dammini* (Acari: Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, USA. J Med Entomol 21:52–57, 1984
39. Magnarelli LA, Anderson JF, Chappell WA: Antibodies to spirochetes in white-tailed deer and prevalence of infected ticks from foci of Lyme disease in Connecticut. J Wildl Dis 20:21–26, 1984